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RICO DEVELOPMENT CORPORATION

WASTEWATER BIOMONITORING

Results of tests completed on December 18, 1991

Prepared for

Rico Development Corporation
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December 18, 1991

SUMMARY

Wastewater from Rico Development Corporation showed both acute and chronic toxicity to Ceriodaphnia, but no acute toxicity to Fathead minnows.

METHODS

A two species acute and a one species chronic bioassay were conducted with wastewater from Rico Development Corporation. A sample of final effluent was composited for eight hours ending on December 8, 1991, and shipped to Commercial Testing and Engineering (CT&E) on December 9, 1991.

A grab sample of final effluent was collected on December 9, 1991, and shipped to CT&E that day. Unfortunately, United Parcel Service misdelivered both the grab and composite samples, and CT&E was unable to receive them until December 11, 1991.

The grab sample was assigned CT&E number 156348 and used for the two species acute test. The composite sample was assigned CT&E number 156347 and used for days 0, 1 and 2 of the chronic test. Subsequent samples of effluent were composited on December 10 and 11 and delivered to CT&E on December 12, 1991.

Testing was conducted in accordance with the most recent protocols specified by the Water Quality Control Division of the Colorado Department of Health (1991), outlined in Tables 1 and 2, and by Region VIII of the U.S. Environmental Protection Agency (USEPA, 1985, 1989 and 1990).

Basic characteristics of the effluent and receiving water were measured including pH, dissolved oxygen, alkalinity, hardness, ammonia and residual free chlorine, as shown in Table 3.

Alkalinity and hardness were determined by titrimetric methods specified by the American Public Health Association (1976). Dissolved oxygen was measured with a YSI model 54A oxygen meter.

The pH was measured using a Fisher Scientific model 910 pH meter and ammonia was measured using the same pH meter with an Orion model 95-12 ammonia-specific electrode. Total residual chlorine was measured using a LaMotte model STC-Cl colorimeter.

The acute tests consisted of exposing the Cladoceran Ceriodaphnia dubia and the Fathead minnow, Pimephales promelas, to five concentrations of effluent, 100%, 72%, 44%, and 22%, prepared using water reconstituted in the laboratory.

The chronic test exposed Ceriodaphnia dubia to only a 44% wastewater concentration. Control replicates in both the acute and chronic tests exposed the test organisms to 100% laboratory reconstituted water.

Colorado law defines acute toxicity as a statistically significant difference, at the 95% confidence level, between mortality in the control and in any effluent concentration equal to or less than the instream waste concentration.

Chronic toxicity is defined as a statistically significant difference, at the 95% confidence level, between Ceriodaphnia reproduction or Fathead weight gain in the control and in any effluent concentration.

Ceriodaphnia survival and reproduction and Fathead survival and weight gain data was evaluated using the Shapiro-Wilk's and Chi square tests of normality and the Hartley and Bartlett's tests of homogeneity of variance.

Steel's Many-One Rank non-parametric test would have been performed on data from the chronic test, which failed to meet the assumptions of normality and homogeneity of variance, to identify significant differences in Ceriodaphnia reproduction, but 100% mortality in all effluent concentrations made statistical analysis of neonate production impossible.

Survival data from acute tests was used to calculate an LC_{50} value, the concentration of an effluent that would be lethal to 50% of test organisms in the duration of an acute test, using the Binomial, Moving Average and Probit methods, as shown in Appendix A.

RESULTS

Water Chemistry

The waters' chemical characteristics appear in Table 3.

Toxicity Tests

The sample showed both acute and chronic toxicity to Ceriodaphnia, but no acute toxicity to Fathead minnows, as shown in Tables 4 and 5.

DISCUSSION

The results of these tests support the decision that has been made by the Rico Development Corporation to initiate a Toxicity Reduction Evaluation early in 1992.

REFERENCES

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1976. Standard Methods for the Examination of Water and Wastewater, 14th ed.. American Public Health Association, Washington, DC.

Colorado Department of Health. 1991. Guidelines for Conducting Whole Effluent Toxicity Tests. Water Quality Control Division, 4210 E. 11th Ave., Denver, Colorado 80220-3716.

United States Environmental Protection Agency. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

United States Environmental Protection Agency. 1988. Region VIII NPDES Whole Effluent Toxics Control Program. July 1 revision. USEPA Region VIII Water Management Division, Denver, CO.

United States Environmental Protection Agency. 1989. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, Cincinnati, OH., 45268.

Table 1. Colorado Acute Toxicity Test Conditions.

	Ceriodaphnia	Fathead Minnows
Test type:	Static Renewal	Static Renewal
Temperature:	20 +/- 1 °C	20 +/- 1 °C
Photoperiod:	16h light/ 8h dark	16h light/ 8h dark
Test Chamber size:	30 ml	600 ml
Test Sample vol.:	15 ml	150 ml
Renewal Period:	24 hours	24 hours
Age of Organisms:	<24 hours	$\leq$ 168 $\pm$ 12 hours
Number per Chamber:	5	5
Replicate Chambers/Conc.:	4	4
Feeding Regime/Chamber:	None	0.1 ml brine shrimp at 0 and 48 hours
Test Duration:	48 hours	96 hours
Dilution Water:	Receiving Water	Receiving Water

Table 2. Colorado Chronic Toxicity Test Conditions.

	Ceriodaphnia	Fathead Minnows
Test type:	Static Renewal	Static Renewal
Temperature:	25 +/- 1 °C	25 +/- 1 °C
Photoperiod:	16h light/ 8h dark	16h light/ 8h dark
Test Chamber size:	30 ml	200 ml
Test Sample vol.:	15 ml	150 ml
Renewal Period:	24 hours	24 hours
Age of Organisms:	<24 hours	≤ 7 days, born within 24 hours
Number per Chamber:	1	10
Replicate Chambers/ Conc.:	10	4
Feeding Regime/ Chamber:	0.1 ml Algae + 0.1 ml YCT/day	0.15 ml brine shrimp twice/day at 6 hour intervals
Test Duration:	Until 60% of control animals have 3 broods	7 days

Table 3. Initial Water Chemistry Measurements.

Parameter	Pond 8 Composite Sample	Dolores River
pH (std. units)	7.7	---[1]
Alkalinity (mg/L CaCO3)	162	---[1]
Hardness (mg/L CaCO3)	843	204
Dissolved Oxygen (mg/L)	6.9	---[1]
Conductivity (us/cm)	---[1]	---[1]
Ammonia (mg/L as N)	<0.1	---[1]
Residual Chlorine (mg/L)	<0.1	---[1]

---[1] = Not Measured

Table 4. Ceriodaphnia and Fathead Minnow Survival Data for Acute Toxicity Tests of sample 1563482132.

	Number Exposed		Number Dead		Percent Mortality	
Conc. (%)	Cerio.	FHM	Cerio.	FHM	Cerio.	FHM
100	20	20	20	1	100.00	5.00
72	20	20	20	0	100.00	0.00
44	20	20	20	0	100.00	0.00
22	20	20	19	0	95.00	0.00
0	20	20	0	0	0.00	0.00

Table 5. Results of the Chronic Toxicity Test of sample 156347.

Exposure	Ceriodaphnia dubia	
Effluent (%)	Mortality (%)	Neonates (mean)
0	0	27.4
44	100	-

APPENDIX A
Statistical analyses applied to data
from acute tests of sample 156348.

12-9-91 Rico CDA
Program run on 12-18-1991 at 12:40:11

RESULTS CALCULATED USING THE BINOMINAL METHOD

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	20	100.00	LESS THAN 0.001
72	20	20	100.00	LESS THAN 0.001
44	20	20	100.00	LESS THAN 0.001
22	20	19	95.00	0.0020

AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST
SHOWS THAT THE LC50 IS ABOVE 22

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.
<37 TO>63 OF PERCENT DEAD

NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN
USING THE NUMBER ALIVE INSTEAD OF THE NUMBER DEAD.

WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE
REASONABLE.

12-9-91 Rico FHA
Program run on 12-18-1991 at 12:47:39

RESULTS CALCULATED USING THE BINOMINAL METHOD

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	1	5.00	0.0020
72	20	0	0.00	LESS THAN 0.001
44	20	0	0.00	LESS THAN 0.001
22	20	0	0.00	LESS THAN 0.001

AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST
SHOWS THAT THE LC50 IS ABOVE 100

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.
<37 TO>63 OF PERCENT DEAD

NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN
USING THE NUMBER ALIVE INSTEAD OF THE NUMBER DEAD.

WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE
REASONABLE.

APPENDIX B
Forms to report results of toxicity tests of samples 156347 and 156348 to the Colorado Department of Health.

COLORADO - CDPS WET TEST REPORT FORM - ACUTE

PERMITTEE: Rico Development Corporation CDPS NO. CO-00 29793 OUTFALL: _____

TYPE TEST: ROUTINE: X ACCELERATED: ___ TEST SPECIES: Ceriodaphnia dubia AGE: <24h.

TEST RESULTS: IWC: 44 % CONTROL MORTALITY: 10 % LC50: <22 %

50% MORTALITY LIMIT: PASS/FAIL CONC WITH STAT. SIGNIFICANT MORTALITY: 22, 44, 72+100 %

SAMPLE TYPE: GRAB/COMPOSITE TIME & DATE: 0930 AM/PM 12-9-91

TEST TIME & DATE: BEGIN 1900 AM/PM 12/11/91 END 0800 AM/PM 12/13/91

DILUTIONS (% EFFLUENT)

MEASUREMENTS	CONTROL(0%)	22%	44%	72%	100%	%
NO. @ START OF TEST:	20	20	20	20	20	
NO. LIVE AFTER 24 HRS:	20	19	0	0	0	
AFTER 48 HRS:	18	1	0	0	0	
AFTER 72 HRS:						
AFTER 96 HRS:						
MAX/MIN VALUES DISSOLVED OXYGEN, MG/L:	7.5/6.6	7.0/6.6	7.5/7.0	7.9/7.0	8.1/7.4	/__
TEMPERATURE °C:	25.0/24.0	25.0/24.0	25.0/24.0	25.0/24.0	25.0/24.0	/__

RECEIVING WATER USED FOR DILUTION? YES/NO SAMPLE AERATED? YES/NO

HARDNESS, MG/L: RECEIVING WATER 204 EFFLUENT ~~850~~ 845 RECON/LAB WATER 78

ALKALINITY, MG/L: RECEIVING WATER _____ EFFLUENT 162 RECON/LAB WATER 65

PH: INITIAL - CONTROL 8.2 100% 7.3 FINAL - CONTROL 8.2 100% 8.5

T. AMMONIA AS N, MG/L: INITIAL - 100% <0.1 FINAL - 100% _____

TOT. RESID. CHLORINE, MG/L: 100% <0.1 SAMPLE DECHLORINATED BEFORE TEST? YES/NO

LABORATORY: Commercial Testing and Engineering Company ANALYST: Fred Offenhartz

COMMENTS: _____

COLORADO - CDPS WET TEST REPORT FORM - ACUTE

PERMITTEE: Pico Development Corporation CDPS NO. CO-0029793 OUTFALL: 002

TYPE TEST: ROUTINE: ✓ ACCELERATED: ___ TEST SPECIES: Pimephales promelas AGE: 24 h.

TEST RESULTS: IWC: 44% CONTROL MORTALITY: 0% LC50: >100%

50% MORTALITY LIMIT: PASS/FAIL CONC WITH STAT. SIGNIFICANT MORTALITY: None %

SAMPLE TYPE: GRAB/COMPOSITE TIME & DATE: 0930 AM/PM 12-9-91

TEST TIME & DATE: BEGIN 1500 AM/PM 12/12/91 END 0800 AM/PM 12/16/91

DILUTIONS (% EFFLUENT)

MEASUREMENTS	CONTROL (0%)	22%	44%	72%	100%	___%
NO. @ START OF TEST:	10	10	10	10	10	
NO. LIVE AFTER 24 HRS:					9	
AFTER 48 HRS:						
AFTER 72 HRS:						
AFTER 96 HRS:						

MAX/MIN VALUES

DISSOLVED OXYGEN, MG/L: 6.8/6.4 7.0/6.5 7.1/6.5 7.2/6.6 7.3/6.5 ___/___

TEMPERATURE °C: 26.0/24.2 26.0/24.2 26.0/24.2 26.0/24.2 26.0/24.2 ___/___

RECEIVING WATER USED FOR DILUTION? YES/NO SAMPLE AERATED? YES/NO

HARDNESS, MG/L: RECEIVING WATER 204 EFFLUENT ~~824~~ 843A RECON/LAB WATER 78

ALKALINITY, MG/L: RECEIVING WATER ___ EFFLUENT 162 RECON/LAB WATER 65

PH: INITIAL - CONTROL 8.2 100% 7.2 FINAL - CONTROL 8.0 100% 8.6

T. AMMONIA AS N, MG/L: INITIAL - 100% <0.1 FINAL - 100% ___

TOT. RESID. CHLORINE, MG/L: 100% <0.1 SAMPLE DECHLORINATED BEFORE TEST? YES/NO

LABORATORY: Commercial Testing and Engineering Co. ANALYST: Fred Offenbacher

COMMENTS: ___

COLORADO - CDPS WET TEST REPORT FORM - CHRONIC

PERMITTEE: Fire Development Corporation CDPS NO. CO-0025793 OUTFALL: 002

TEST SPECIES: Ceriodaphnia dubia AGE: <24h. RECEIVING WATER USED FOR DILUTION? YES (NO)

TEST RESULTS: CONTROL MORTALITY: 10 % CONTROL PERFORMANCE: $\bar{x}$= 27.44

CONC. WITH STAT. SIG.: MORTALITY: 44% % REPRODUCTION see below % GROWTH _____ %

SAMPLE TYPE: GRAB/(COMPOSITE)

TEST:

	TIME & DATE		TIME & DATE
NO. 1	0900 AM/PM 12/8/91	BEGINNING	1845 AM/PM 12/11/91
NO. 2	0830 AM/PM 12/10/91	ENDING	1300 AM/PM 12/18/91
NO. 3	0730 AM/PM 12/11/91	SPECIAL CONDITION: SAMPLE AERATED?	YES/(NO)

DILUTIONS (% EFFLUENT)

MEASUREMENTS	CONTROL (0%)	44 %	_____ %	_____ %	_____ %	_____ %
% SURVIVAL FOR DAY: 1	100	40				
2		0				
3						
4	90					
5						
6						
7						
MEAN 3 BROOD TOTAL:	27.4					
7 DAY MEAN DRY WEIGHT:						
D.O., MG/L MAX/MIN:	7.5/6.3	7.3/6.7	___/___	___/___	___/___	___/___
TEMP °C MAX/MIN:	26.0/24.0	25.0/24.0	___/___	___/___	___/___	___/___
PH MAX/MIN:	8.3/8.0	8.4/7.2	___/___	___/___	___/___	___/___

HARDNESS ($\bar{x}$) MG/L RECEIVING WATER: 204 EFFLUENT: 843 RECON/LAB WATER: 78

ALKALINITY ($\bar{x}$) MG/L RECEIVING WATER: _____ EFFLUENT: 162 RECON/LAB WATER: 65

T. AMMONIA as N ($\bar{x}$) MG/L INITIAL EFFLUENT: <0.1 FINAL EFFLUENT: _____

T. RESIDUAL CHLORINE, MG/L 100%: <0.1 SAMPLE DECHLORINATED BEFORE TEST? YES/(NO)

LABORATORY: Commercial Testing and Engineering Company ANALYST: Fred Offenbacher

1/1/91